# CLAIMS

What is claimed is:

1. A method for assaying for an amplification product from a first target polynucleotide, comprising:

providing a sample that is suspected of containing the amplification product, wherein the amplification product is a polynucleotide comprising a first label and a capture sequence not present in the target polynucleotide at the same position, wherein the amplification product is formed by primer extension from a template, wherein said template comprises a complement to the target polynucleotide and a target noncomplementary region, wherein said capture sequence is a complement to said target noncomplementary region;

providing a substrate that is conjugated to a first capture probe;

contacting the sample with the capture probe under a first set of hybridization conditions;

wherein the capture probe can bind to the capture sequence under said first set of hybridization conditions; and

determining if the first label is associated with the substrate.

2. The method of claim 1, wherein the capture probe is a polynucleotide.

3. The method of claim 2, wherein the substrate is selected from the group consisting of a microsphere, a chip, a slide, a multiwell plate, a membrane, an optical fiber, and an optionally porous gel matrix.

4. The method of claim 3, wherein the substrate is a slide.

5. The method of claim 2, wherein the substrate is conjugated to a plurality of different capture probe polynucleotides having corresponding different sequences, wherein each of said different capture probes can selectively bind to a corresponding different capture sequence on a corresponding different amplification product.

6. The method of claim 3, wherein the substrate is a first microsphere comprising a first spectral code comprising a first semiconductor nanocrystal and first fluorescence characteristics.

7. The method of claim 6, wherein the first semiconductor nanocrystal comprises a core selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaSe, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, Ge, Si, Pb, PbS, PbSe, an alloy thereof, and a mixture thereof.

8. The method of claim 7, wherein the core is CdSe.

9. The method of claim 6, wherein the first semiconductor nanocrystal comprises a shell.

10. The method of claim 9, wherein the shell is ZnS.

11. The method of claim 2, wherein the amplification product is produced by a process comprising incorporation of a nucleotide comprising the first label into the amplification product using a polymerase.

12. The method of claim 2, wherein the amplification product is produced by a process comprising extension of a primer using a polymerase to form the amplification product, said primer comprising the first label.

13. The method of claim 2, wherein the first label comprises an agent selected from a chromophore, a lumiphore, a fluorophore, a chromogen, a hapten, an antigen, a radioactive isotope, a magnetic particle, a metal nanoparticle, an enzyme, an antibody or binding portion or equivalent thereof, an aptamer, and one member of a binding pair.

14. The method of claim 13, wherein the agent is a fluorophore.

15. The method of claim 14, wherein the fluorophore is selected from a semiconductor nanocrystal, a fluorescent dye, a lanthanide chelate, and a green fluorescent protein.

16. The method of claim 15, wherein the fluorophore is a semiconductor nanocrystal.

17. The method of claim 16, wherein the semiconductor nanocrystal comprises a core selected from the group consisting of ZnS, ZnSe, ZnTe, CdS, CdSe, CdTe, HgS, HgSe, HgTe, MgS, MgSe, MgTe, CaS, CaSe, CaTe, SrS, SrSe, SrTe, BaS, BaSe, BaTe, GaN, GaP, GaAs, GaSb, InN, InP, InAs, InSb, AlAs, AlP, AlSb, AlS, Ge, Si, Pb, PbS, PbSe, an alloy thereof, and a mixture thereof.

18. The method of claim 17, wherein the core is CdSe.

19. The method of claim 16, wherein the semiconductor nanocrystal comprises a shell.

20. The method of claim 19, wherein the shell is ZnS.

21. The method of claim 15, wherein the fluorophore is a fluorescent dye.

22. The method of claim 21, wherein the fluorescent dye is fluorescein.

23. The method of claim 15, wherein the fluorophore is a lanthanide chelate selected from a europium chelate, a terbium chelate and a samarium chelate.

24. The method of claim 13, wherein the agent is an enzyme selected from alkaline phosphatase, horseradish peroxidase, β-galactosidase, glucose oxidase, a bacterial luciferase, an insect luciferase and sea pansy luciferase.

25. The method of claim 13, wherein the agent is selected from avidin, streptavidin, digoxigenin, and biotin.

26. The method of claim 2, wherein the first label is a fluorophore, and determining if the first label is associated with the substrate comprises:

applying a light source to the substrate that can excite the fluorophore; and

determining if a fluorescence emission from the fluorophore occurs from the substrate.

27. The method of claim 1, wherein the sample is assayed for the presence of the amplification product.

28. The method of claim 1, wherein the sample is assayed to determine the amount of the amplification product.

29. The method of claim 28, wherein the amplification product is produced at a detectably higher level from at least one allele of a locus having at least two alleles.

30. The method of claim 6, wherein the sample is suspected of containing a second amplification product from a second target polynucleotide and is further contacted under a second set of hybridization conditions with a second capture probe conjugated to a second microsphere,

wherein the second capture probe is a polynucleotide,

wherein the second microsphere can be the first microsphere or a different second microsphere,

wherein when the second microsphere is a different second microsphere it comprises a second spectral code comprising second fluorescence characteristics, said second spectral code distinguishable from the first spectral code,

wherein the second set of hybridization conditions can be the same as or different than the first set of hybridization conditions,

wherein the second capture probe can hybridize to the second amplification product under the second set of hybridization conditions,

wherein the second amplification product comprises a second label, which can be the first label when the second microsphere is a different second microsphere or can be a different second label, and

determining if the second label is associated with the second microsphere.

31. The method of claim 30, wherein the sample is suspected of containing a third amplification product from a third target polynucleotide and is further contacted under a third set of hybridization conditions with a third capture probe conjugated to a third microsphere,

wherein the third capture probe is a polynucleotide,

wherein the third microsphere can be the first microsphere, the second microsphere or a different third microsphere,

wherein when the third microsphere is a different third microsphere it comprises a third spectral code comprising third fluorescence characteristics, said third spectral code distinguishable from the first spectral code and the second spectral code,

wherein the third set of hybridization conditions can be the first set of hybridization conditions, the second set of hybridization conditions, or a different third set of hybridization conditions,

wherein the third capture probe can hybridize to the third amplification product under the third set of hybridization conditions,

wherein the third amplification product comprises a third label, which can be the first label or the second label when the third microsphere is a different third microsphere or can be a different third label, and

determining if the third label is associated with the third microsphere.

32. The method of claim 31, wherein the sample is suspected of containing a fourth amplification product from a fourth target polynucleotide and is further contacted under a fourth set of hybridization conditions with a fourth capture probe conjugated to a fourth microsphere,

wherein the fourth capture probe is a polynucleotide,

wherein the fourth microsphere can be the first microsphere, the second microsphere, the third microsphere or a different fourth microsphere,

wherein when the fourth microsphere is a different fourth microsphere it comprises a fourth spectral code comprising fourth fluorescence characteristics, said fourth spectral code distinguishable from the first spectral code, the second spectral code and the third spectral code,

wherein the fourth set of hybridization conditions can be the first set of hybridization conditions, the second set of hybridization conditions, the third set of hybridization conditions or a different fourth set of hybridization conditions,

wherein the fourth capture probe can hybridize to the fourth amplification product under the fourth set of hybridization conditions,

wherein the fourth amplification product comprises a fourth label, which can be the first label, the second label or the third label when the fourth microsphere is a different fourth microsphere or can be a different fourth label, and

determining if the fourth label is associated with the fourth microsphere.

33. The method of claim 30, wherein the first and second amplification products are produced from a single locus.

34. The method of claim 33, wherein the first and second amplification products differ by a single nucleotide.

35. The method of claim 30, wherein the second microsphere is the first microsphere and both first and second capture probes are conjugated to the first microsphere, and wherein the first and second labels are fluorophores comprising distinguishable fluorescence characteristics.

36. The method of claim 30, wherein the second microsphere is a different second microsphere, and wherein the first and second labels each comprise the same fluorophore.

37. The method of claim 30, wherein the second microsphere is a different second microsphere, and wherein the first and second labels respectively comprise first and second fluorophores having distinguishable fluorescence characteristics.

38. The method of claim 1, wherein the substrate is further conjugated to a second capture probe, wherein the second capture probe can preferentially bind to a second capture sequence on a second amplification product, said second amplification product comprising a second label that can be the same as or different than the first label, wherein the binding of the first amplification product to the first capture probe and of the second amplification product to the second capture probe can be independently determined.

39. The method of claim 1, wherein the substrate is further conjugated to a third capture probe, wherein the third capture probe can preferentially bind to a third capture sequence on a third amplification product, said third amplification product comprising a third label that can be the same as or different than the first label and/or the second label, wherein the binding of the third amplification product to the third capture probe can be independently determined.

40. The method of claim 1, wherein the substrate is further conjugated to a fourth capture probe, wherein the fourth capture probe can preferentially bind to a fourth capture sequence on a fourth amplification product, said fourth amplification product comprising a fourth label that can be the same as or different than the first label and/or the second label and/or the third label, wherein the binding the fourth amplification product to the fourth capture probe can be independently determined.

41. The method of claim 38, wherein the first and second capture probes are conjugated to first and second positions on the substrate, and wherein the binding of the first amplification product to the first capture probe and of the second amplification product to the second capture probe can be independently determined by determining if the first label is associated with the first position and if the second label is associated with the second position.

42. The method of claim 38, wherein the second label is different from the first label, and wherein the binding of the first amplification product to the first capture probe and of the second amplification product to the second capture probe can be independently determined by

determining if the first label is associated with the substrate and if the second label is associated with the substrate.

43. A method of forming an amplification product detection complex for assaying a sample for a first target polynucleotide, comprising:

providing a first primer and a second primer;

said first primer comprising a 3` end, a first target complementary region that is complementary to the first target polynucleotide, said first target complementary region located at the 3` end of the first primer, and a first target noncomplementary region that is not complementary to the first target polynucleotide at a position 3` of a sequence to which the first target complementary region can hybridize;

said second primer comprising a 3` end and a first label;

providing the sample, said sample suspected of containing the first target polynucleotide;

contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first target polynucleotide and the first primer can be extended to form a first primer extension product;

altering the sample conditions to allow dissociation of the first primer extension product from the first target polynucleotide;

wherein the 3` end of the second primer is complementary to the first primer extension product at a position in the first primer extension product that is 3` to the first target complementary region;

contacting the sample with the second primer under conditions in which the second primer can hybridize to the first primer extension product and be extended to form a second primer extension product comprising a first capture sequence that is the complement of the first target noncomplementary region and does not exist elsewhere in the second primer extension product, wherein the second primer extension product is the amplification product;

altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product; and

contacting the sample with a first capture probe conjugated to a first substrate, wherein the contacting takes place under conditions in which the first capture probe can bind to the first

capture sequence of the second primer extension product to form an amplification product detection complex.

44. A method of assaying for an amplification product from a first target polynucleotide comprising performing the method of claim 43, and determining if the first label is associated with the first substrate.

45. The method of claim 44, wherein the first capture probe is a polynucleotide.

46. The method of claim 45, wherein the first substrate is a first microsphere comprising a first spectral code, said first spectral code comprising a first semiconductor nanocrystal and first fluorescence characteristics.

47. The method of claim 43, wherein the amplification product is produced at a detectably higher level from at least one allele of a locus having at least two alleles.

48. The method of claim 46, further comprising identifying the first microsphere by decoding the first spectral code.

49. The method of claim 48, wherein the first spectral code is decoded prior to determining if the first label from the second primer is associated with the substrate.

50. The method of claim 48, wherein the spectral code is decoded simultaneously with determining if the label from the second primer is associated with the substrate.

51. The method of claim 48, wherein the spectral code is decoded subsequent to determining if the label from the second primer is associated with the substrate.

52. The method of claim 43, wherein the first target polynucleotide is DNA.

53. The method of claim 43, wherein the first target polynucleotide is RNA.

54. The method of claim 53, wherein a polymerase having reverse transcriptase activity is used to form the first primer extension product.

55. The method of claim 53, wherein a DNA polymerase is used to form the first primer extension product.

56. The method of claim 43, wherein the first target polynucleotide is single-stranded.

57. The method of claim 43, wherein the first target polynucleotide is double-stranded.

58. The method of claim 43, wherein, after altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product, the sample is further contacted with the first and second primers under conditions in which the first and second primers can hybridize to the second and first primer extension products, respectively, and be extended to form a plurality of first and second primer extension products.

59. The method of claim 43, wherein altering the sample conditions to allow dissociation of the first primer extension product from the first target polynucleotide comprises heating the sample.

60. The method of claim 43, wherein altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product comprises heating the sample.

61. The method of claim 43, further comprising removing single-stranded polynucleotides from the sample prior to altering the sample conditions to allow dissociation of the second primer extension product from the first primer extension product.

62. The method of claim 60, wherein removing single-stranded polynucleotides from the sample comprises adding a thermolabile single-stranded nuclease to the sample under conditions suitable and for a time sufficient to digest single-stranded polynucleotides in the sample, and then heating the sample to inactivate the single-stranded nuclease.

63. The method of claim 43, wherein the first target polynucleotide is at least one allele of a locus comprising a second allele, and wherein the first primer preferentially hybridizes to and is extended from said at least one allele as compared to the second allele.

64. The method of claim 63, wherein one of the first and second primers has at least one mismatch with said second allele at one of the five 3` nucleotides of the first primer.

65. The method of claim 63, wherein said at least one allele and said second allele differ by a single nucleotide polymorphism.

66. The method of claim 63, further comprising contacting the sample with a flanking primer that is complementary to the first target polynucleotide and can be extended to form a flanking primer extension product, wherein said flanking primer is complementary to the first target polynucleotide at a flanking position that is 5` to a position at which the first target complementary region is complementary to the first target polynucleotide.

67. The method of claim 63, wherein the flanking primer has a lower melting point for hybridization to the first target polynucleotide than the first primer.

68. The method of claim 43, said method further comprising forming a second amplification product detection complex for assaying the sample for a second target polynucleotide by:

providing a third primer and a fourth primer;

said third primer comprising a 3` end, a second target noncomplementary region that is not complementary to the second target polynucleotide, and a second target complementary

region that is complementary to the second target polynucleotide, said second target complementary region located at the 3' end of the third primer;

said fourth primer comprising a 3' end and a second label, which may be the first label or a different second label;

said sample suspected of containing the second target polynucleotide;

contacting the sample with the third primer under conditions in which the second target complementary region can hybridize to the second target polynucleotide and the third primer can be extended to form a third primer extension product;

altering the sample conditions to allow dissociation of the third primer extension product from the second target polynucleotide;

wherein the 3' end of the fourth primer is complementary to the third primer extension product at a position in the third primer extension product that is 3' to the second target complementary region;

contacting the sample with the fourth primer under conditions in which the fourth primer can hybridize to the third primer extension product and be extended to form a fourth primer extension product comprising a second capture sequence that is the complement of the second target noncomplementary region, wherein the fourth primer extension product is the second amplification product;

altering the sample conditions to allow dissociation of the fourth primer extension product from the third primer extension product;

providing a second capture probe conjugated to a second substrate, which may be the first substrate or a different second substrate; and

contacting the sample with the second substrate under conditions in which the second capture probe can bind to the second capture sequence of the fourth primer extension product to form a second amplification product detection complex.

69. The method of claim 68, wherein the second substrate is a different second substrate, and the first substrate is a first microsphere comprising a first spectral code comprising first fluorescence characteristics, and the second substrate is a second microsphere comprising a second spectral code comprising second fluorescence characteristics.

70. The method of claim 68, said method further comprising forming a third amplification product detection complex for assaying the sample for a third target polynucleotide by:

providing a fifth primer and a sixth primer;

said fifth primer comprising a 3` end, a third target noncomplementary region that is not complementary to the third target polynucleotide, and a third target complementary region that is complementary to the third target polynucleotide, said third target complementary region located at the 3` end of the fifth primer;

said sixth primer comprising a 3` end and a third label, which may be the first label, the second label or a different third label;

said sample suspected of containing the third target polynucleotide;

contacting the sample with the fifth primer under conditions in which the third target complementary region can hybridize to the third target polynucleotide and the fifth primer can be extended to form a fifth primer extension product;

altering the sample conditions to allow dissociation of the fifth primer extension product from the third target polynucleotide;

wherein the 3` end of the sixth primer is complementary to the fifth primer extension product at a position in the fifth primer extension product that is 3` to the third target complementary region;

contacting the sample with the sixth primer under conditions in which the sixth primer can hybridize to the fifth primer extension product and be extended to form a sixth primer extension product comprising a third capture sequence that is the complement of the third target noncomplementary region, wherein the sixth primer extension product is the third amplification product;

altering the sample conditions to allow dissociation of the sixth primer extension product from the fifth primer extension product;

providing a third capture probe conjugated to a third substrate, which may be the first substrate, the second substrate or a different third substrate; and

contacting the sample with the third substrate under conditions in which the third capture probe can bind to the third capture sequence of the sixth primer extension product to form a third amplification product detection complex.

71. The method of claim 70, wherein the second substrate is a different second substrate and the third substrate is a different third substrate, wherein the first substrate is a first microsphere comprising a first spectral code comprising first fluorescence characteristics, wherein the second substrate is a second microsphere comprising a second spectral code comprising second fluorescence characteristics, and wherein the third substrate is a third microsphere comprising a third spectral code comprising third fluorescence characteristics.

72. The method of claim 70, said method further comprising forming a fourth amplification product detection complex for assaying the sample for a fourth target polynucleotide by:

providing a seventh primer and a eighth primer;

said seventh primer comprising a 3` end, a fourth target noncomplementary region that is not complementary to the fourth target polynucleotide, and a fourth target complementary region that is complementary to the fourth target polynucleotide, said fourth target complementary region located at the 3` end of the seventh primer;

said eighth primer comprising a 3` end and a fourth label, which may be the first label, the second label, the third label or a different fourth label;

said sample suspected of containing the fourth target polynucleotide;

contacting the sample with the seventh primer under conditions in which the fourth target complementary region can hybridize to the fourth target polynucleotide and the seventh primer can be extended to form a seventh primer extension product;

altering the sample conditions to allow dissociation of the seventh primer extension product from the fourth target polynucleotide;

wherein the 3` end of the eighth primer is complementary to the seventh primer extension product at a position in the seventh primer extension product that is 3` to the fourth target complementary region;

contacting the sample with the eighth primer under conditions in which the eighth primer can hybridize to the seventh primer extension product and be extended to form an eighth primer extension product comprising a fourth capture sequence that is the complement of the fourth target noncomplementary region, wherein the eighth primer extension product is the fourth amplification product;

altering the sample conditions to allow dissociation of the eighth primer extension product from the seventh primer extension product;

providing a fourth capture probe conjugated to a fourth substrate, which may be the first substrate, the second substrate, the third substrate or a different fourth substrate; and

contacting the sample with the fourth substrate under conditions in which the fourth capture probe can bind to the fourth capture sequence of the eighth primer extension product to form a fourth amplification product detection complex.

73. The method of claim 71, wherein the second substrate is a different second substrate and the third substrate is a different third substrate and the fourth substrate is a different fourth substrate, wherein the first substrate is a first microsphere comprising a first spectral code comprising first fluorescence characteristics, wherein the second substrate is a second microsphere comprising a second spectral code comprising second fluorescence characteristics, and wherein the third substrate is a third microsphere comprising a third spectral code comprising third fluorescence characteristics, and wherein the fourth substrate is a fourth microsphere comprising a fourth spectral code comprising fourth fluorescence characteristics.

74. The method of claim 68, wherein the second substrate is a different second substrate.

75. The method of claim 68, wherein the second substrate is the first substrate.

76. The method of claim 68, wherein the first and second labels have the same fluorescence characteristics.

77. The method of claim 68, wherein the first and second labels have distinguishable fluorescence characteristics.

78. The method of claim 68, wherein the first target polynucleotide is at least a first allele and the second target polynucleotide is at least a second allele of a locus.

79. The method of claim 78, wherein the first and second target polynucleotides differ by a single nucleotide polymorphism.

80. A method of assaying a sample for a first target polynucleotide, comprising:

providing a first primer and a second primer;

said first primer comprising a 3` end, a first target complementary region that is complementary to the first target polynucleotide, said first target complementary region located at the 3` end of the first primer, and a first target noncomplementary region that is not complementary to the first target polynucleotide at a position 3` of a sequence to which the first target complementary region can hybridize;

said second primer comprising a 3` end and a first label;

providing the sample, said sample suspected of containing the first target polynucleotide;

contacting the sample with the first primer under conditions in which the first target complementary region can hybridize to the first target polynucleotide and the first primer can be extended to form a first primer extension product;

heating the sample to allow dissociation of the first primer extension product from the first target polynucleotide;

wherein the 3` end of the second primer is complementary to the first primer extension product at a position in the first primer extension product that is 3` to the first target complementary region;

contacting the sample with the second primer under conditions in which the second primer can hybridize to the first primer extension product and be extended to form a second primer extension product comprising a first capture sequence that is the complement of the first

target noncomplementary region and does not exist elsewhere in the second primer extension product, wherein the second primer extension product is the amplification product;

heating the sample to allow dissociation of the second primer extension product from the first primer extension product;

contacting the sample with a first capture probe polynucleotide conjugated to a substrate, wherein the contacting takes place under conditions in which the first capture probe can hybridize to the first capture sequence of the second primer extension product;

wherein the first label is a fluorophore or is linked to a fluorophore;

applying a light source to the substrate that can excite the fluorophore; and

determining if a fluorescence emission from the fluorophore occurs from the substrate.

81. An amplification product detection complex comprising a capture probe polynucleotide hybridized to the capture sequence of a labeled amplification product from a target polynucleotide,

wherein the capture probe polynucleotide is conjugated to a substrate,

wherein the capture sequence is not present in a region of the target polynucleotide which is amplified and is introduced into the amplification product by copying a template polynucleotide,

said template polynucleotide comprising a target noncomplementary region and a region complementary to the target polynucleotide,

wherein said target noncomplementary region was introduced into the template polynucleotide by extension of a primer hybridized to said target polynucleotide, said primer comprising said target noncomplementary region, and

wherein said capture sequence is complementary to said target noncomplementary region.

82. The amplification product detection complex of claim 81, wherein the capture probe polynucleotide is conjugated at a 3' end to the substrate.

83. The amplification product detection complex of claim 81, wherein the capture probe is conjugated at a 5' end to the substrate.

84. The amplification product detection complex of claim 81, wherein the capture probe is conjugated at an internal position to the substrate.

85. The amplification product detection complex of claim 81, wherein the substrate is a microsphere comprising a spectral code comprising a semiconductor nanocrystal.

86. A kit for assaying for an amplification product from a target polynucleotide comprising:

a substrate attached to a capture probe;

a first primer comprising a 3' end, a first target complementary region that is complementary to the first target polynucleotide, said first target complementary region located at the 3' end of the first primer, and a first target noncomplementary region that is not complementary to the first target polynucleotide at a position 3' of a sequence to which the first target complementary region can hybridize;

a second primer;

a label;

a housing for retaining the substrate, first primer, second primer and label; and

instructions provided with said housing that describe how to use the components of the kit to assay a sample by forming an amplification product from the target polynucleotide using the first and second primers that comprises the label and a capture sequence that is complementary to the target noncomplementary region in the first primer, wherein said capture sequence can bind to the capture probe.

87. The kit of claim 86, wherein the label is provided conjugated to a nucleotide which can be incorporated into the amplification product.

88. The kit of claim 86, wherein the label is provided conjugated to the second primer.

89. The kit of claim 86, wherein the substrate is attached to a plurality of different capture probes, wherein each of said different capture probes is attached at an identifiable location on the substrate, wherein each of said different capture probes can preferentially hybridize to a corresponding different amplification product, each of said corresponding different amplification products comprising a label that can be the same or different as the label on the other corresponding different amplification products, and

wherein said instructions further describe how to use the components of the kit to assay the sample for each of said corresponding different amplification products.